
DTU

Simulated exercise on CPO outbreak – *Acinetobacter baumannii*

EURGen-RefLabCap
Virtual multidisciplinary training workshop – session 2 (06-02-2024)
January-February 2024

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Agenda for today

- **Scenario Background (Faisal)**
  - Overview of *A. baumannii* study in Denmark
  - Scenario
  - Injects

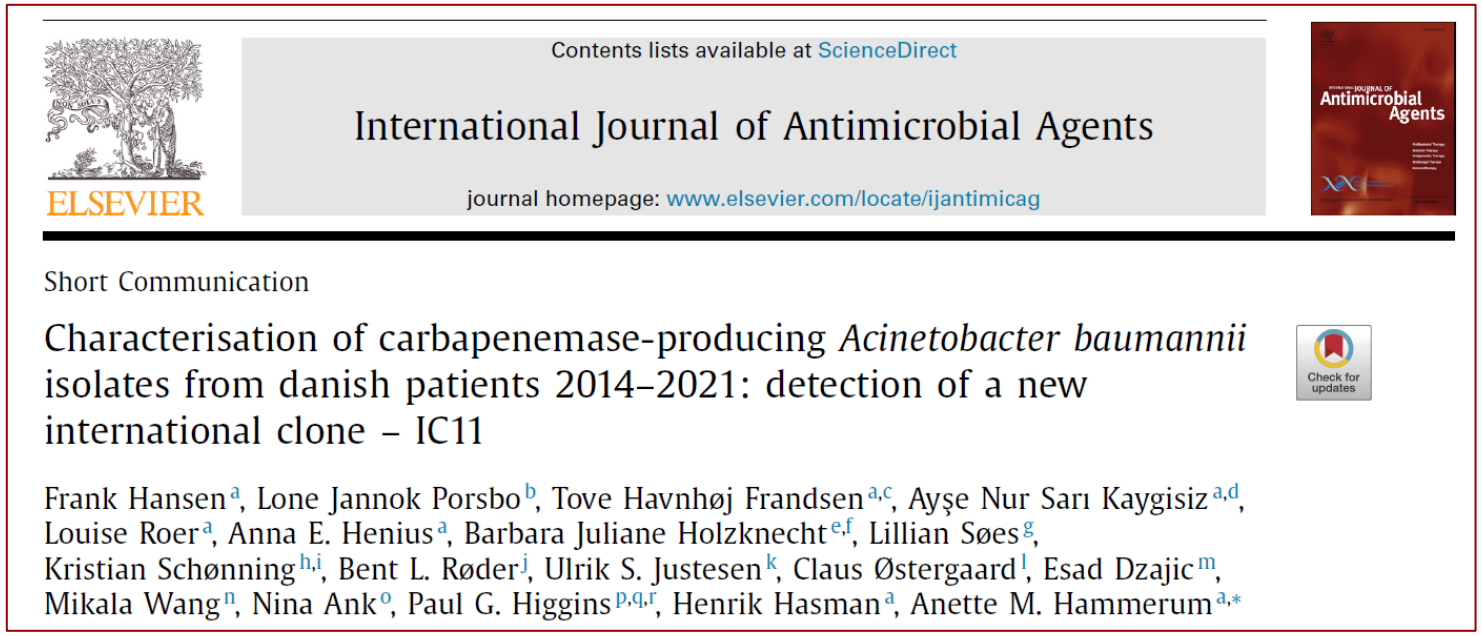
- **Presentation of exercise results (outbreak investigation)**
  - Survey 1
  - Survey 2

- **Discussion regarding epidemiological and genetic analysis for outbreak investigation**

- **Questions/comments from participants**

Scenario Background

- The sequencing data was taken from *A. baumannii* study in Denmark - 2023



Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Short Communication

Characterisation of carbapenemase-producing *Acinetobacter baumannii* isolates from danish patients 2014–2021: detection of a new international clone – IC11

Frank Hansen[a], Lone Jannok Porsbo[b], Tove Havnhøj Frandsen[a,c], Ayşe Nur Sarı Kaygisiz[a,d], Louise Roer[a], Anna E. Henius[a], Barbara Juliane Holzknecht[e,f], Lillian Søes[g], Kristian Schønning[h,i], Bent L. Røder[j], Ulrik S. Justesen[k], Claus Østergaard[l], Esad Dzajic[m], Mikala Wang[n], Nina Ank[o], Paul G. Higgins[p,q,r], Henrik Hasman[a], Anette M. Hammerum[a,*]

https://doi.org/10.1016/j.ijantimicag.2023.106866

- Sequencing data from the study are available in GenBank **PRJEB60981**.

International clones of *A. baumannii*

- 11 international clones
  - 110/141 isolates belonged to IC2
  - *bla*$_{OXA-23}$ most prevalent (n=116)
  - IC11 was first described in this study

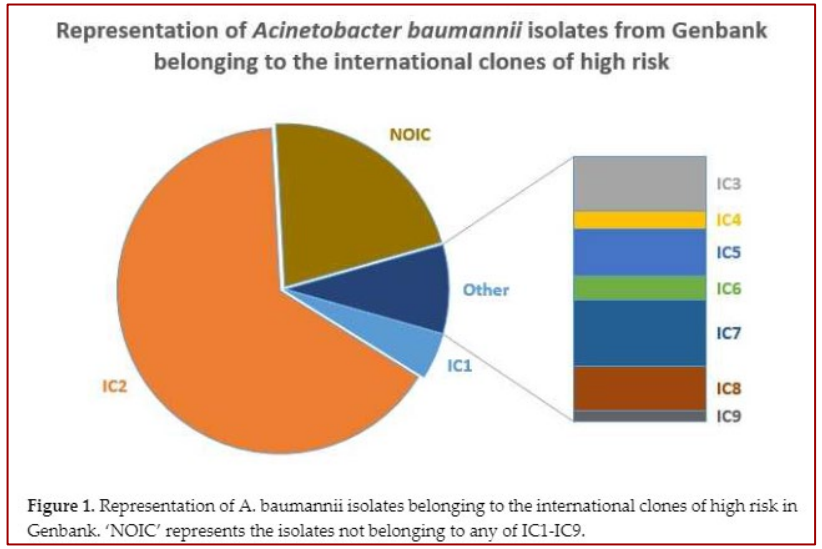
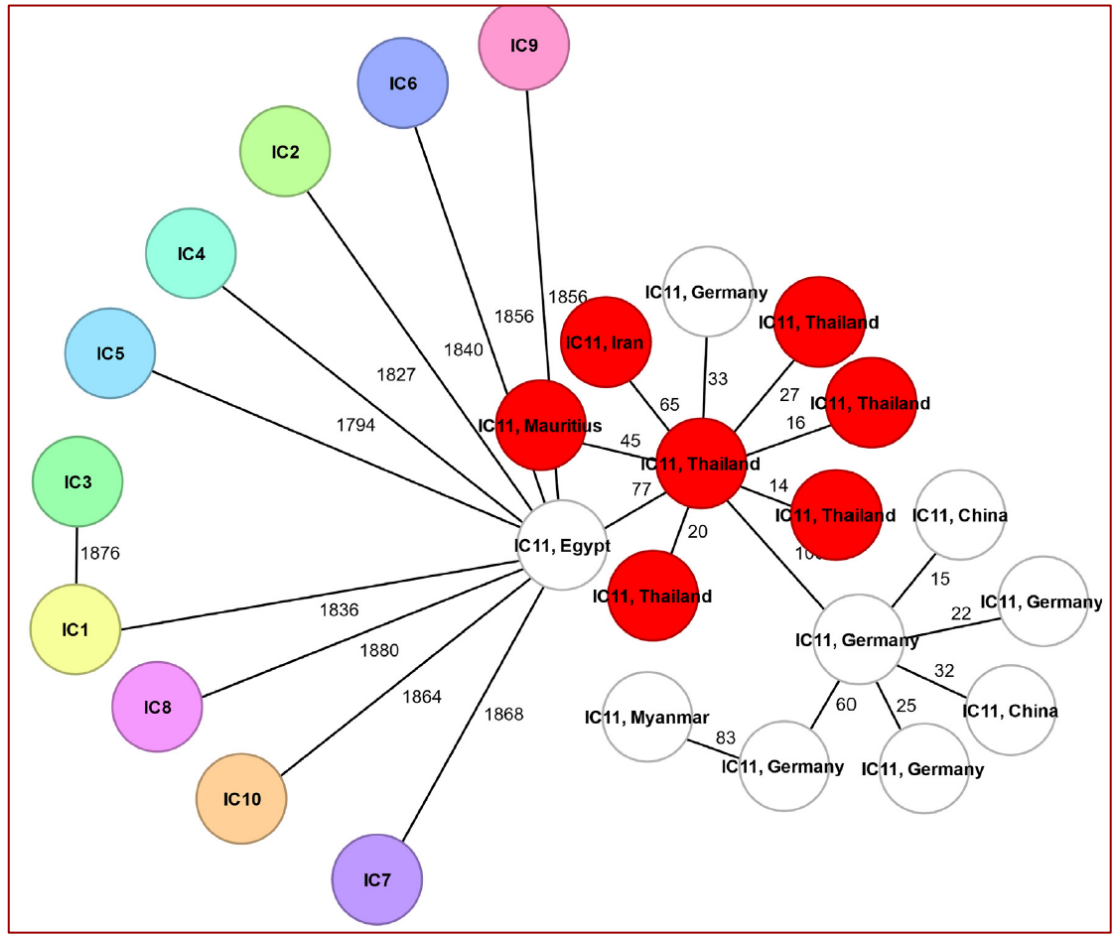


Figure 1. Representation of A. baumannii isolates belonging to the international clones of high risk in Genbank. 'NOIC' represents the isolates not belonging to any of IC1-IC9.

http://dx.doi.org/10.20944/preprints202311.1869.v1



https://doi.org/10.1016/j.ijantimicag.2023.106866

Scenario

- An increase in the number of *A. baumannii* infections observed in the country during 2023
- The NRL obtained the isolates and associated metadata from 6 hospitals (n=78)
- The NRL wants to do a pilot study and sequence 35 isolates

- **Objective**: To investigate possible outbreak clusters of *A. baumannii* from six hospitals

Inject 1.2 (Patient metadata)

- **Aim**: To generate EpiCurve and select isolates for sequencing

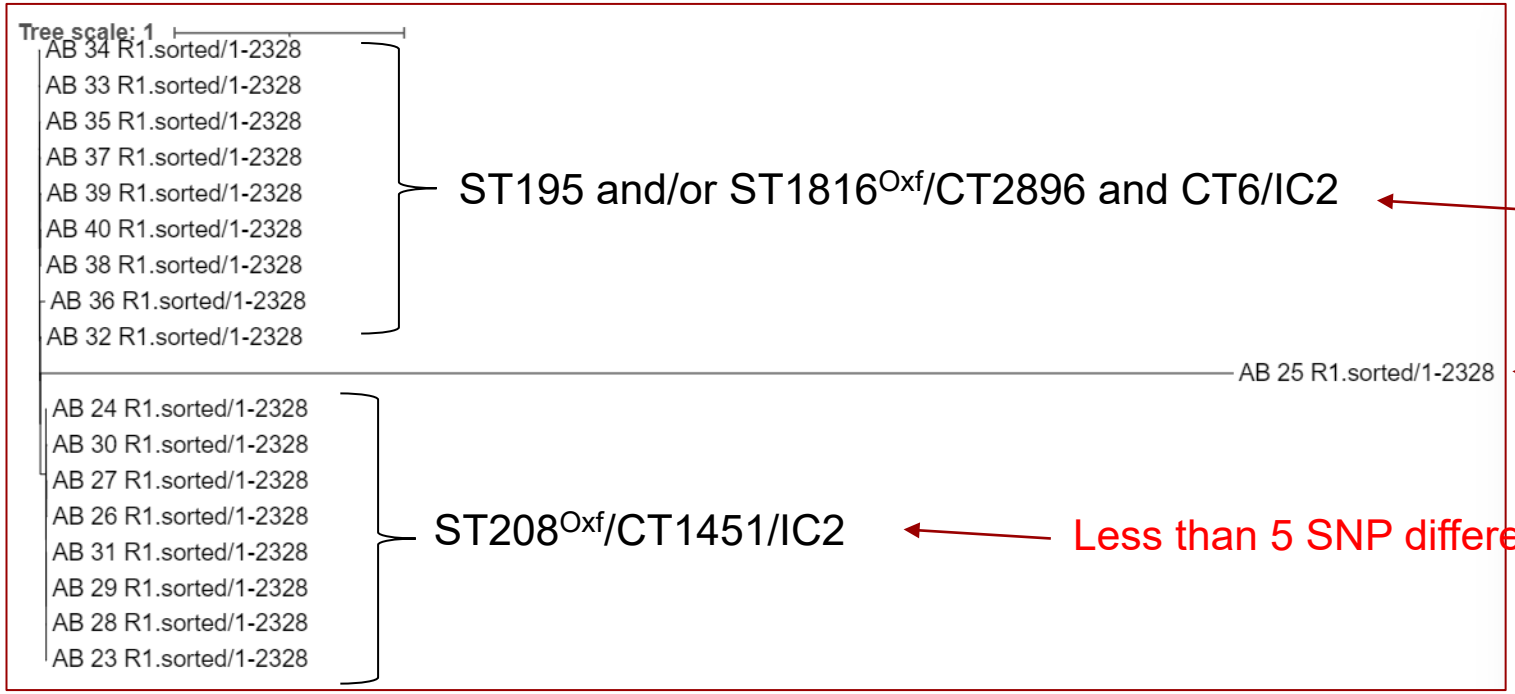
- Patient metadata was provided for 78 *A. baumannii* isolates
  - Admission/Discharge date
  - Symptoms onset/sampling date
  - Antibiogram/PCR results for carbapenemase gene

- A case was defined as a patient with a clinical or screening sample positive for carbapenem resistant *A. baumannii*.

- EpiCurve: An increased number of cases are observed in July/August
  - AB_23 to AB_58

Inject 2.1 (Isolates from Hospital A)

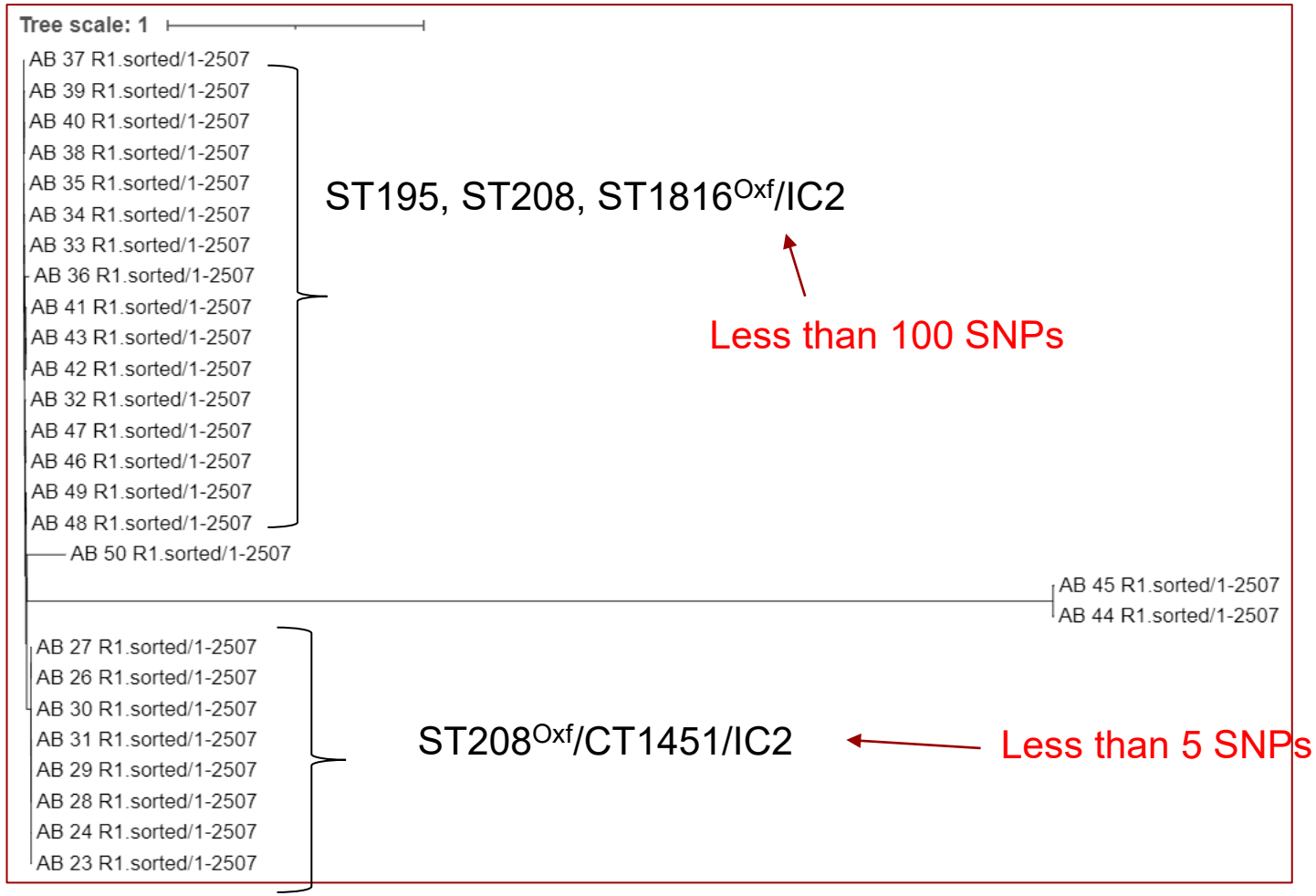
- Two obvious clusters
- AB_25 is very distant and can be safely removed from further analysis



Inject 3.1 (Hospital B and C)

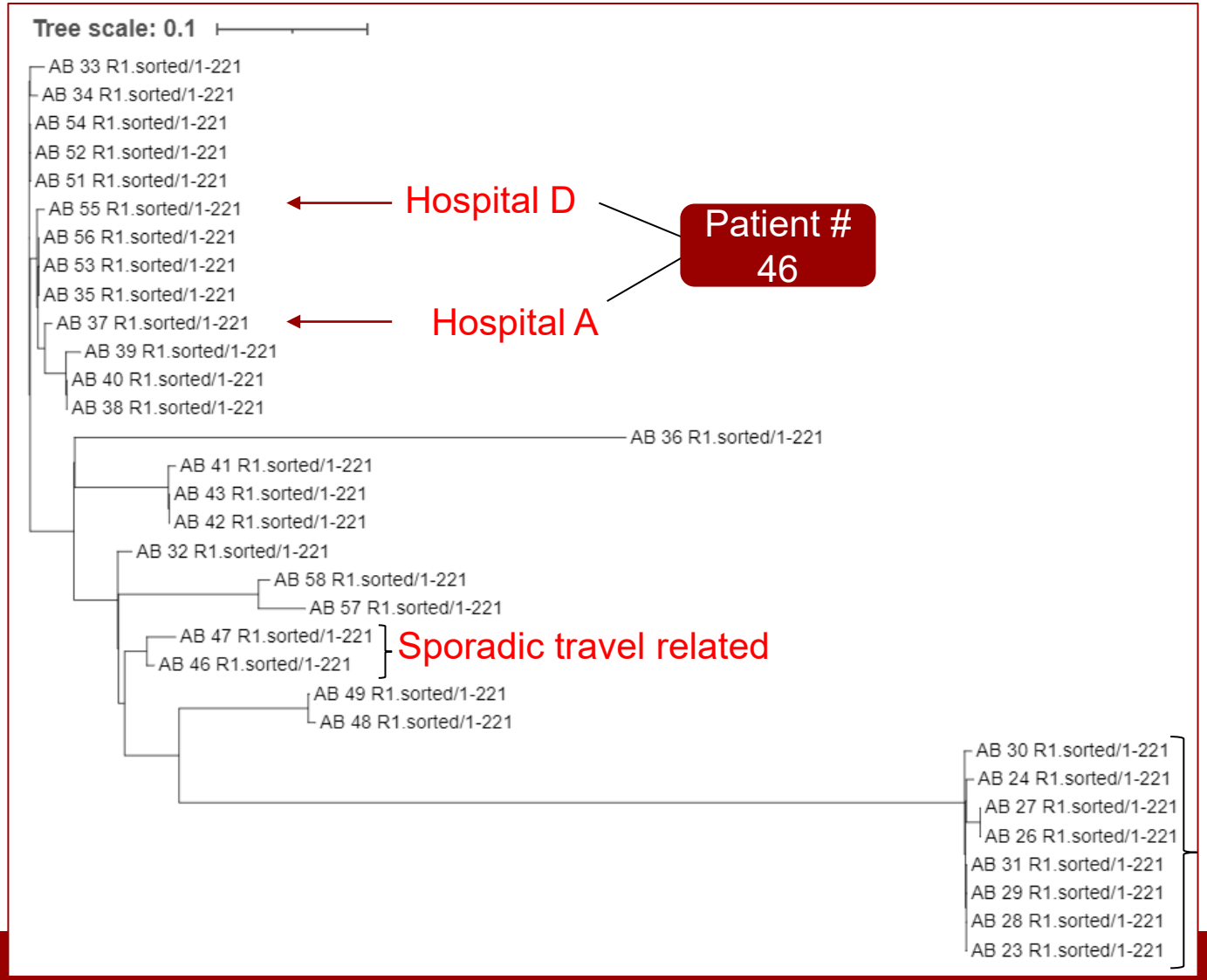
- Hospital B and C isolates cluster closely with Hospital A isolates
  - E.g., AB_42 from hospital B vs AB_33
  - AB_47 from hospital C vs AB_33

- AB_44, AB_45, and AB_50 can be removed in further analysis



Inject 3.2 (Hospital D, E, and F)

- Several clusters and subclusters
- Confirmation of inter-hospital transmission
  - Patient #46: AB_37 vs AB_55
  - Transferred from Hosp. D to Hosp. A
- Sporadic travel related
  - AB_46 and AB_47 (Country Z)



Survey 1 questions

- Thank you all for participating with survey answers!
  - 24 responses
  - 19 countries
- Questions related to epi-data
- Focus on how/why to select isolates for sequencing

EURGen RefLabCap virtual multidisciplinary training workshop January 2024 Survey 1

Dear member of the EURGen-RefLabCap network,

Thank you for participating in the virtual multidisciplinary training workshop on simulated outbreak exercises on carbapenem-resistant *Acinetobacter baumannii*, January-February 2024. This is the first of two results' surveys that we invite participants to fill out, in order to evaluate the overall understanding of the outbreak analysis exercises, and to identify areas that we need to give more attention in the second online meeting. The questions in this survey relate only to Inject 1.2 (Sample metadata), and the deadline for submitting answers is **Friday 26th January 12:00 CET.**

Contact person's full name

Country

Institute

Data Inject 1.2

Exercise Background

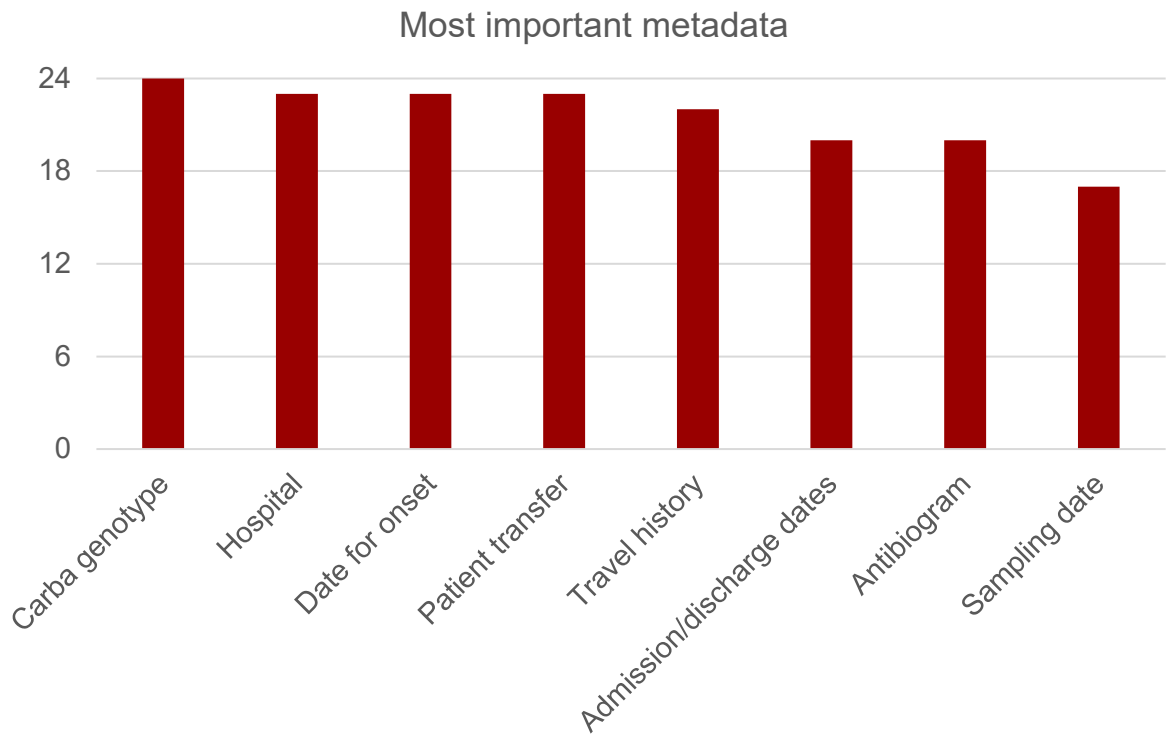
In the recent year (2023), an increasing number of *A. baumannii* infections has been observed and referred to the National Reference Laboratory (NRL). The majority of these are caused by carbapenem resistant *A. baumannii* (CRAB). Several hospitals in different cities have asked for assistance to investigate the possibility of one or more outbreaks, and the NRL has urged hospitals to share epidemiological and patient data of the cases. The NRL also requested the hospitals to send the isolates to the NRL for reference testing and whole genome sequencing (WGS) for the retrospective investigation of possible outbreak(s).

The NRL has received patient records and metadata of 71 cases from 6 hospitals (a total of 78 isolates), including a limited set of phenotypic data, including some antibiograms and PCR confirmation of carbapenemase genes. The NRL does not routinely perform WGS but could select 30-35 bacterial isolates for WGS in a pilot project with a focus on carbapenem resistance. The NRL must decide which isolates to prioritize for the WGS.
Examine the available data (Inject 1.2), consider possible outbreak hypotheses, and answer the following questions:

Q1: Which metadata indicators will you consider when selecting isolates for sequencing? (Multiple selections possible)

Survey 1 Question 1

- **Which metadata indicators will you consider when selecting isolates for sequencing?**



Survey 1 Question 2

- Any additional information missing that is useful for selecting the isolates?

- Ward of hospital
  - Room information
  - Ward type
- Type of samples (clinical vs screening)
- Colonization at admission
- Type of OXA PCR (OXA-group)
- Inter-hospital transfer
- Illness history
- More demographic information:
  - Contact between patients
  - Household connection or travel to same destination
  - Workplace connections…

Discussion points!

- Which kind of data do you get access to as NRL?
- Can you have direct contact with patients?
  - Interview, online questionnaire…?
- Any chance of follow-up?

- How is the amount and quality of data you receive?
  - Perfect world vs real world?

- What can be improved?

Survey 1 Question 3

- Could the metadata immediately suggest any of the below listed outbreak hypotheses?

  – Travel related outbreak(s)

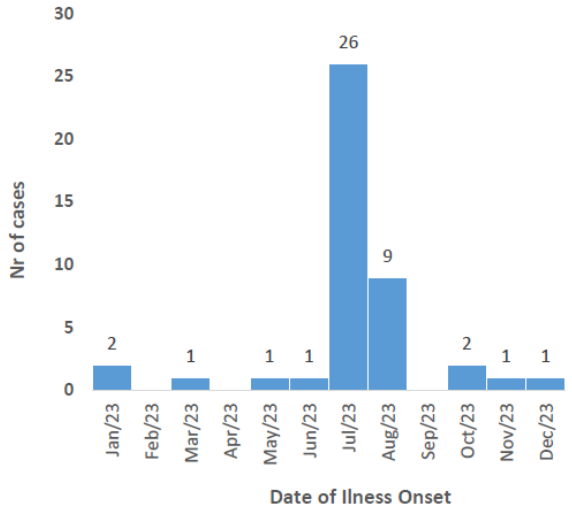
  – Inter-hospital transmission(s)

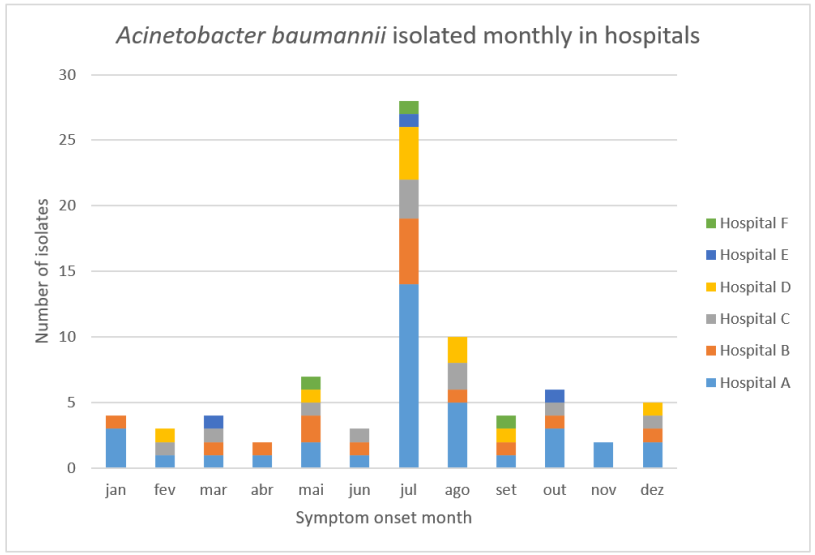
  – Intra-hospital transmission(s) **(1)**

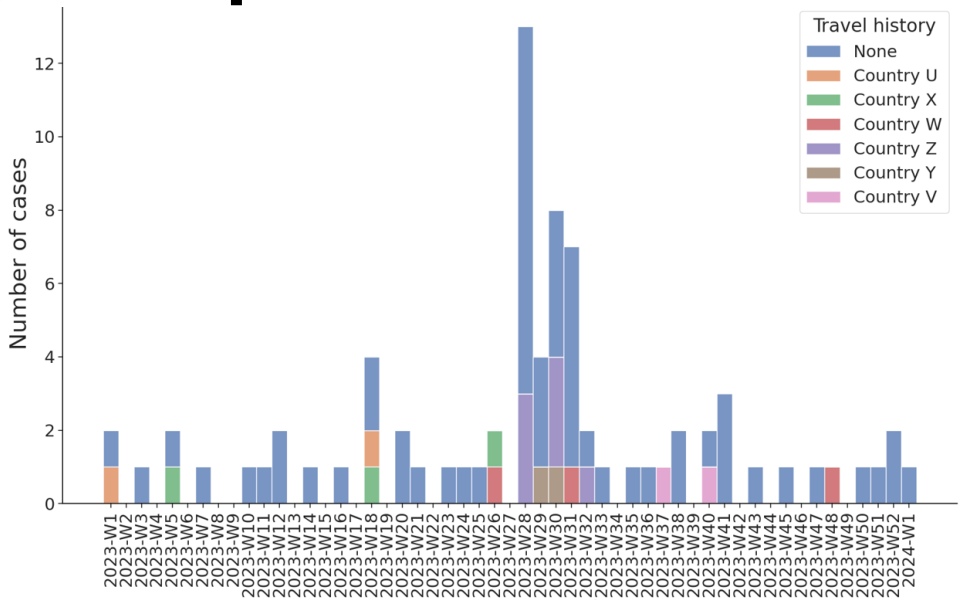
  – **A combination of the above (22)**

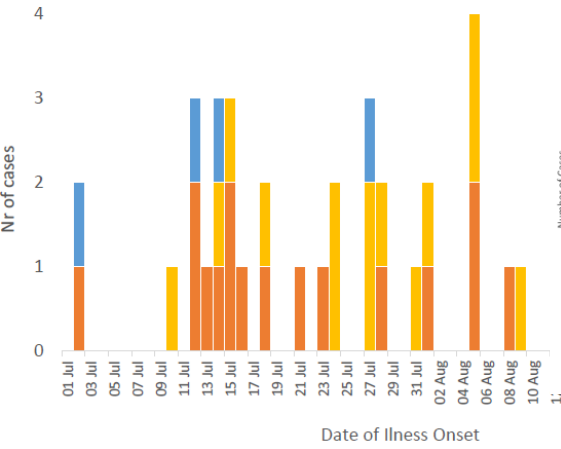
  – Data does not suggest any hypothesis **(1)**

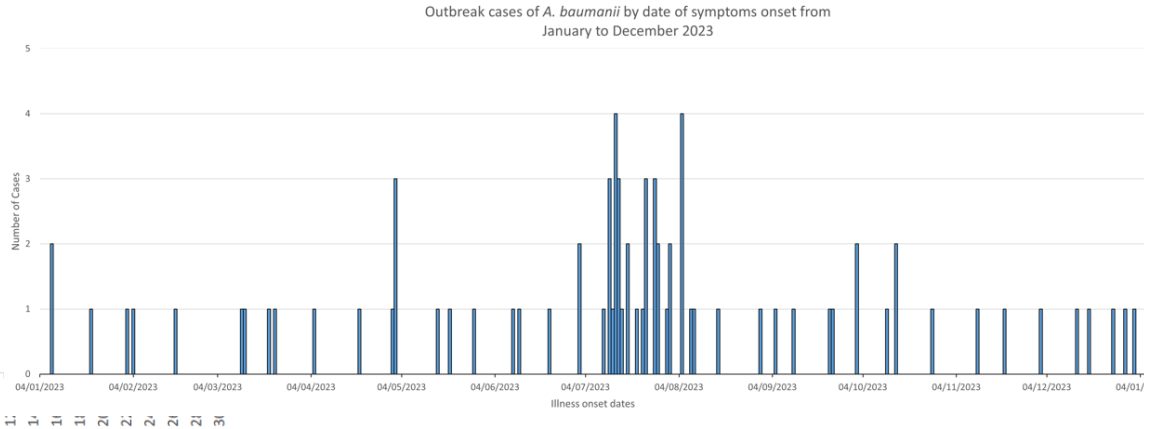
Survey 1 Question 4 – Upload your EpiCurve ☺

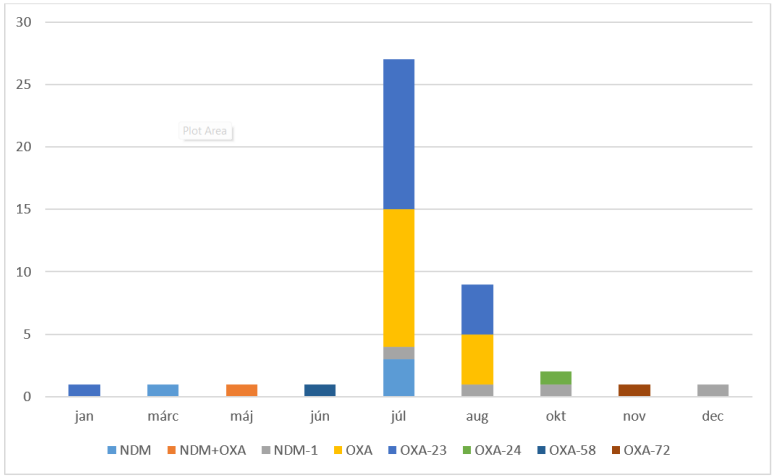






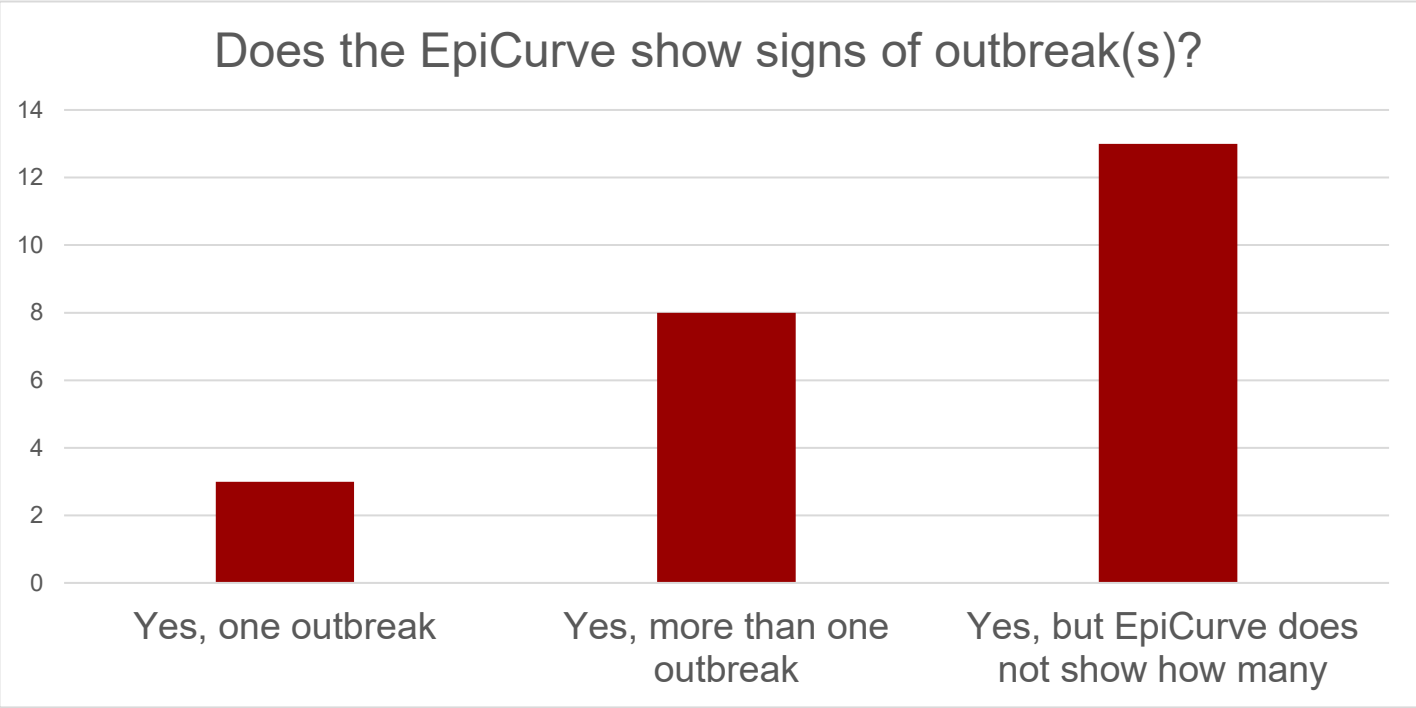






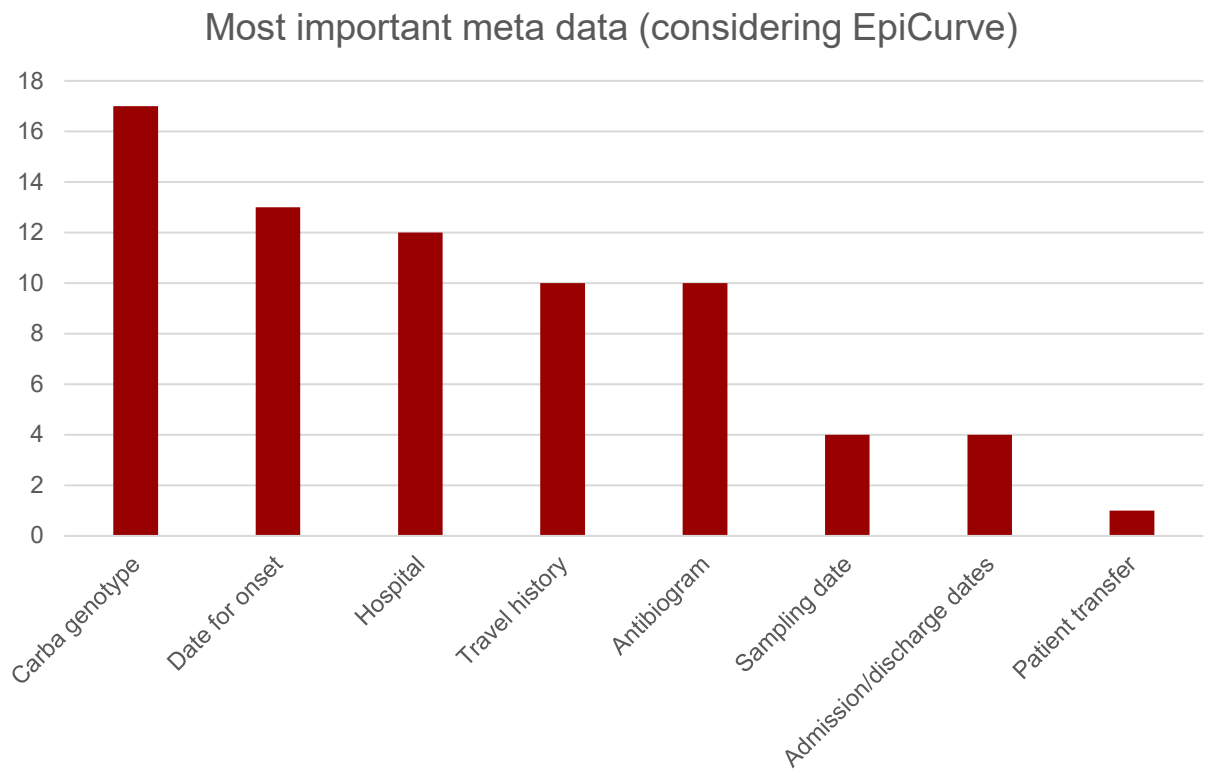
Survey 1 Question 5

- By examining the EpiCurve, does it look like there is an outbreak?
- If yes, does the EpiCurve show how many outbreaks are apparently present over the year 2023?



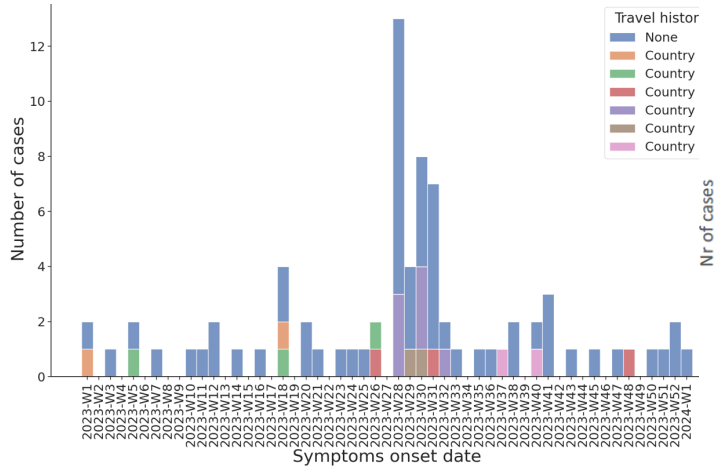
Survey 1 Question 6

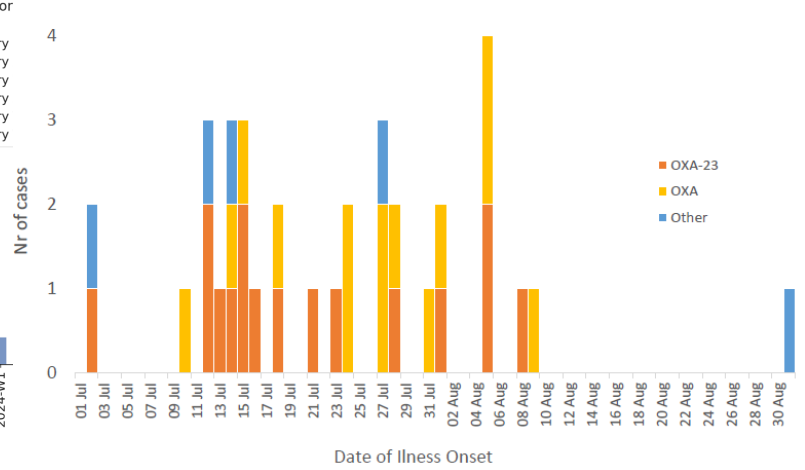
- Considering your EpiCurve, which metadata indicators do you now find most important when selecting isolates for sequencing

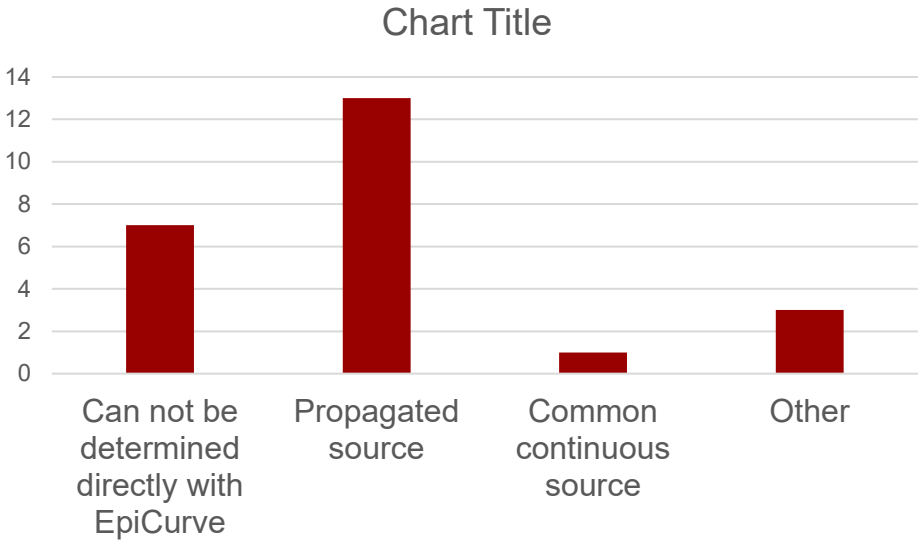


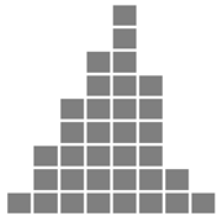
Survey 1
Question 7

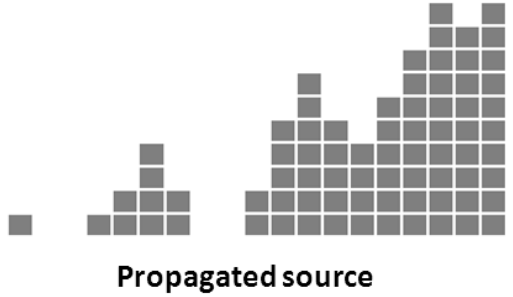
- Here you can see different patterns of EpiCurves and an explanation of how they can be interpreted. Can you tell if the outbreak source appears to be a continuous common source or a progressive source (propagated source) or something else?









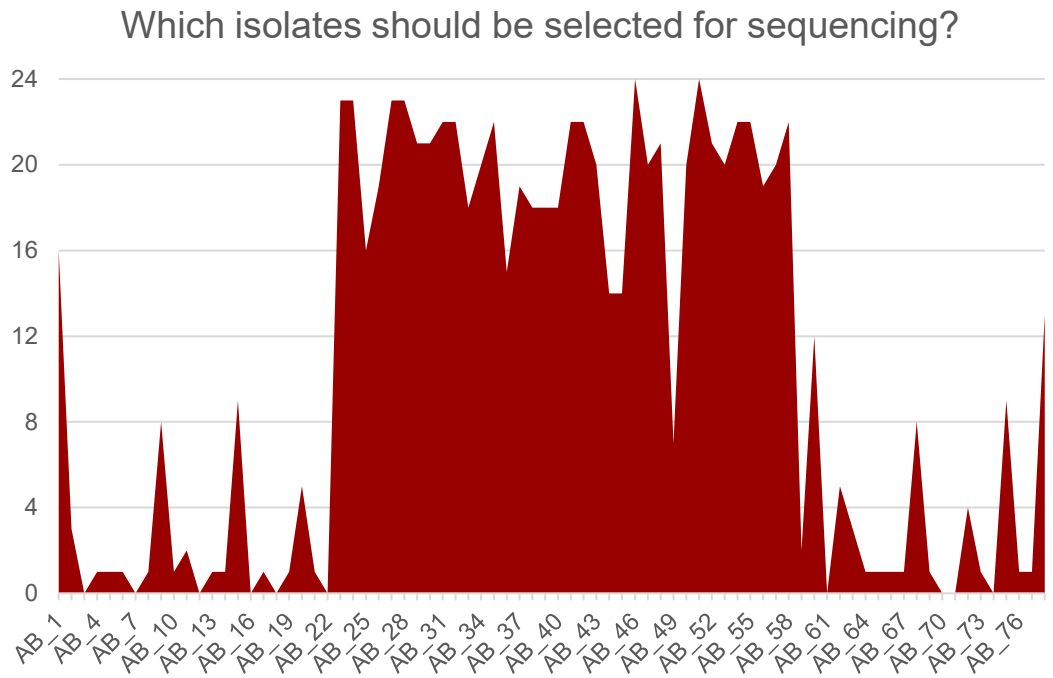


Based on our Epi Curve we assume that there is more then one outbreak, a point source and intermittent source

(Image source: https://outbreaktools.ca/background/epidemic-curves/)

Survey 1 Question 8

- Select the isolates for whole genome sequencing:



Selection criteria:

Primarily: OXA-23, Mero-R and time line.

-Isolates selected based on Mero resistance and OXA-type beta-lactamase by PCR with symptoms onset dates ranging from 02.07.2023 to 27.07.2023

-Signal of NDM positive isolates that could be linked, even though there is no overlap in time of admission (an intermittent source)

-Timeline shows a possible outbreak of AB with OXA-23 at hospital A (hypothesis: from Patient 22 with travel history). From data we can't rule out that P37, P38 and P40 at hospital B belong to the outbreak even though they do not have an overlap to hospital A. Some will not be included based on antibiogram and symptoms onset data, which is > 6 months prior to the other OXA-23 cases at hospital A.

-A group of AB OXA positive samples are identified across all hospitals, and with travel history for > 50% of the patients.

Survey 1 Question 9 - comments

- The results of the antibiotic susceptibility tests were not provided as recommended by EUCAST (e.g. AMP, PIP+TAZO, TET)
  – Was the same panel used for all isolates?

- Isolate carbapenemase genotype had insufficient information regarding OXA PCR (which OXA genes are included - acquired or also intrinsic) - for this reason we have focused on antibiogram for selection.
- It would be possible that additional patients were transferred between hospitals but were not screened for CRAb
- It would be useful to have environmental isolates included (if the environment was sampled).
- Other factors might be present to make this one big outbreak. These can include contaminated supplies common to all hospitals, movement of staff, cleaning materials and possible common cleaning agencies.
- Hypothesis so far is that index case in Hospital A was travel related (AB_23: positive within 2 days from admission) and than patient to patient spread (AB_24, AB_26, etc).

Survey 2

Thank you again for participation!

- 18 participants
- 15 countries

EURGen RefLabCap virtual multidisciplinary training workshop Jan-Feb 2024 Survey 2

Dear member of the EURGen-RefLabCap network,

Part 1 Data Inject 2.1

You have now selected a subset of the *Acinetobacter baumannii* for sequencing and the first batch has been sequenced and analysed. You have received the typing data (MLSTs and cgMLST) and some overall results of SNP analysis, which can be used to further elucidate if there can be identified outbreaks among the *A. baumannii* isolates collected in your country in 2023.
All 'Comment' boxes are optional.

Q1: Looking at the phylogenetic tree and SNP matrix, how would you evaluate the results of the cluster analysis in inject 2.1 regarding data from Hospital A:

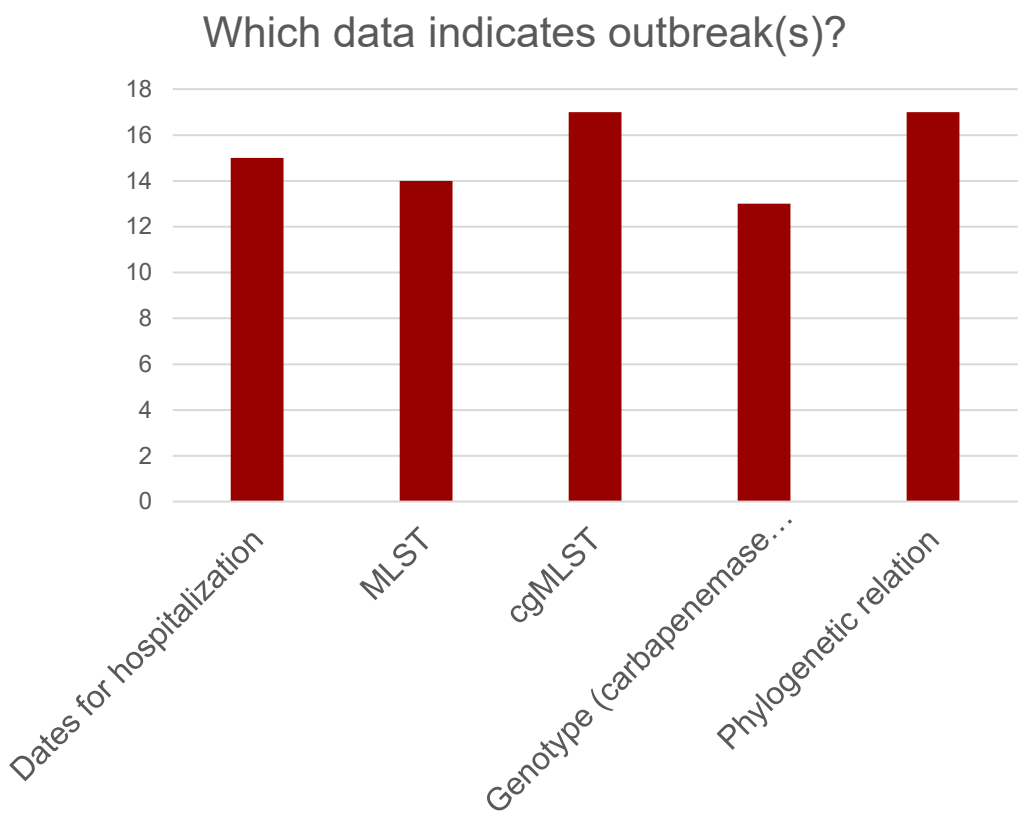
- ☐ No clusters/outbreaks
- ☐ 1 cluster
- ☐ 2 clusters
- ☐ More than 2 clusters
- ☐ Cannot be determined based on the cluster analysis alone

Survey 2: Q1 and 2A/B

- Q1: How many clusters?
- **2 clusters (14)**
- >2 clusters **(2)**
- Cannot be determined on the cluster analysis alone **(2)**

- Q2: Do you see signs of intra-hospital transmission in the Hospital A data, and how?
- **AB_23; AB_24; AB_26; AB_27; AB_28; AB_29; AB_30; AB_31;**
  - AB_32; AB_33; AB_34; AB_35; (AB_36); AB_37; AB_38; AB_39; AB_40

Survey 2 Question 2C



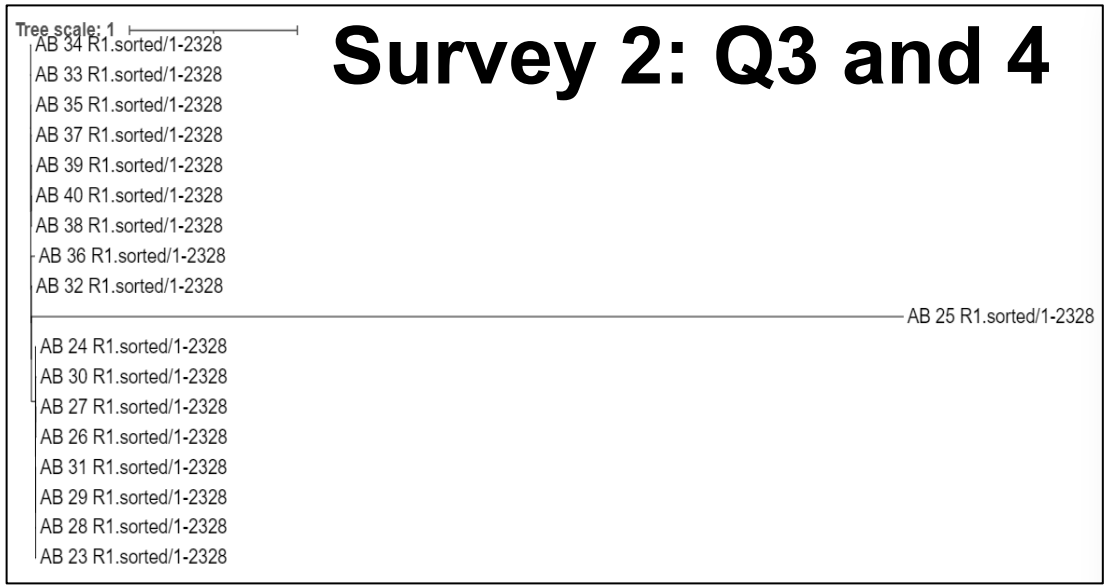
- Cluster I: AB_23-24-26-27-28-29-30-31.
- cgMLST shows allelic difference of 0-3 to the closest neighbour.
- All isolates with the same MLST_pasteur, MLST_Oxford, cgMLST, and carbapenemase gene.
- The Antibiogram shows that AB_26 differs by not showing resistance to GEN, otherwise they are all identical.
- All patients can be directly linked with overlapping admission to hospital A, with sample date and onset of sympoms after connection to another patient.

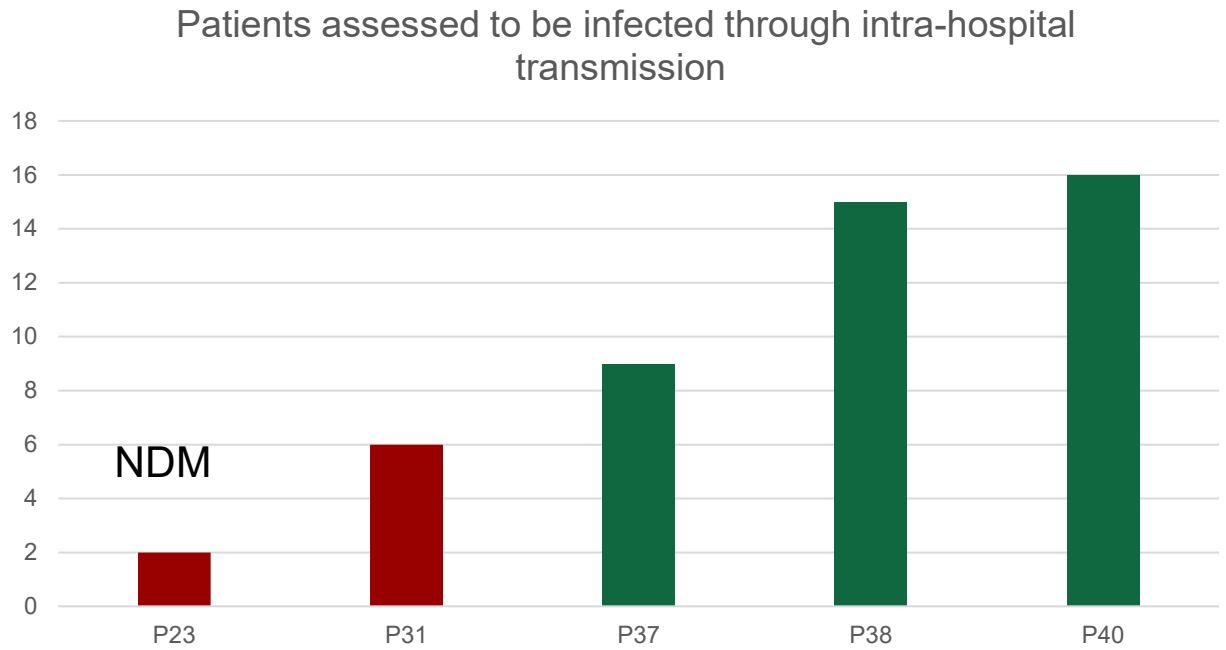
- Cluster II: Ab_33-34-35-37-38-39-40, exhibiting 0-8 SNPs difference within the cluster and an average distance of 65 SNPs from isolates in cluster I.

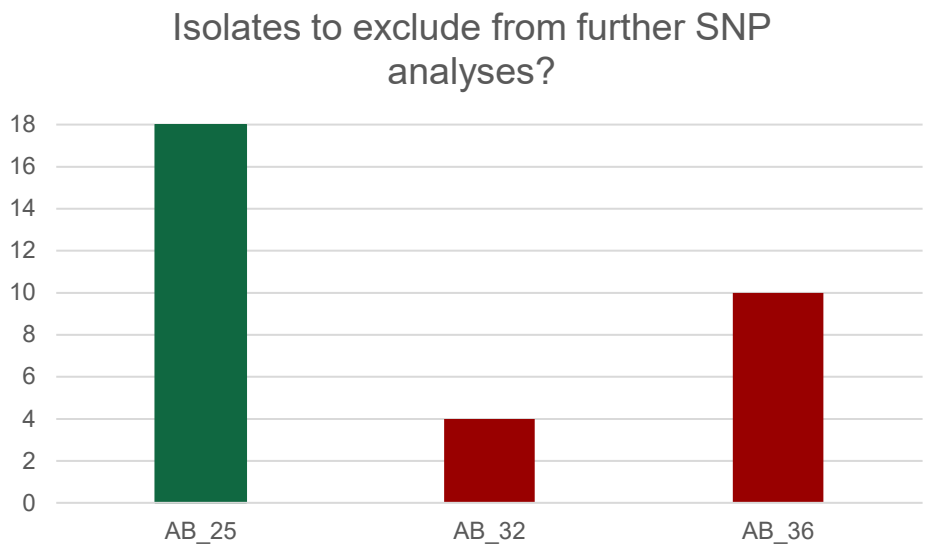
Ab_32 is 11-17 SNPs away

Ab_36 is 44-91 SNPs away and a distinct genotype

Survey 2: Q3 and 4







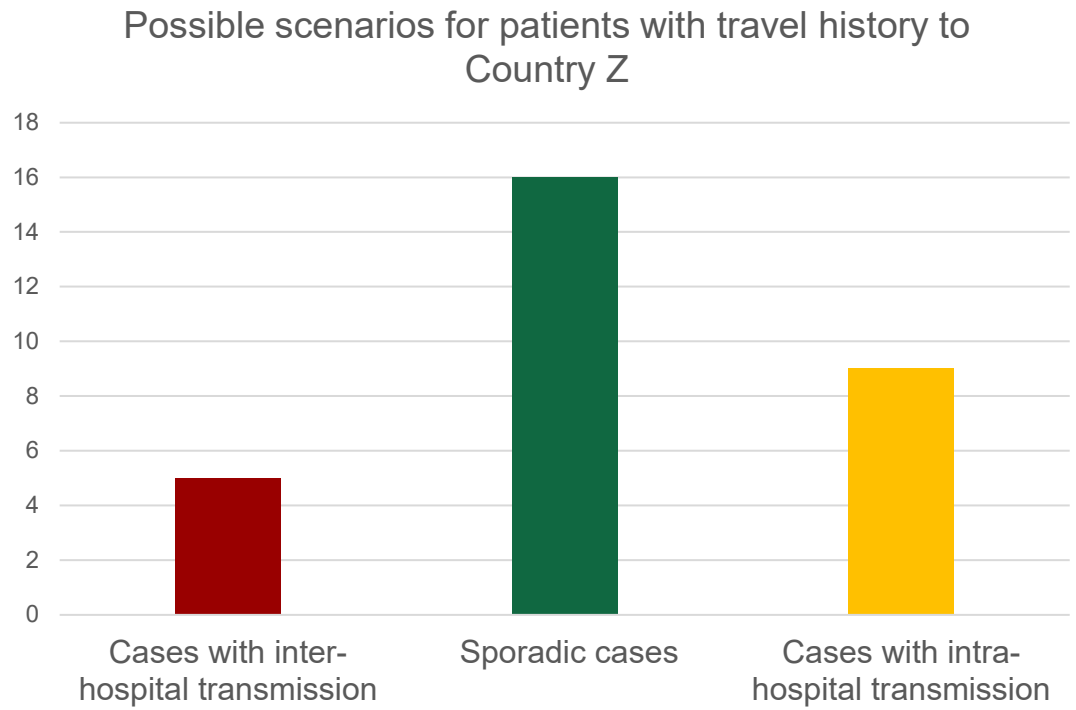
Q4C: Which patient would you select as index patient and why?

AB_23, because it was the first one to present symptoms in this hospital (Patient 22)

Patient No 37 - first admitted case (16.9.2023), positive travel history (AB_37)

Survey 2 Question 6

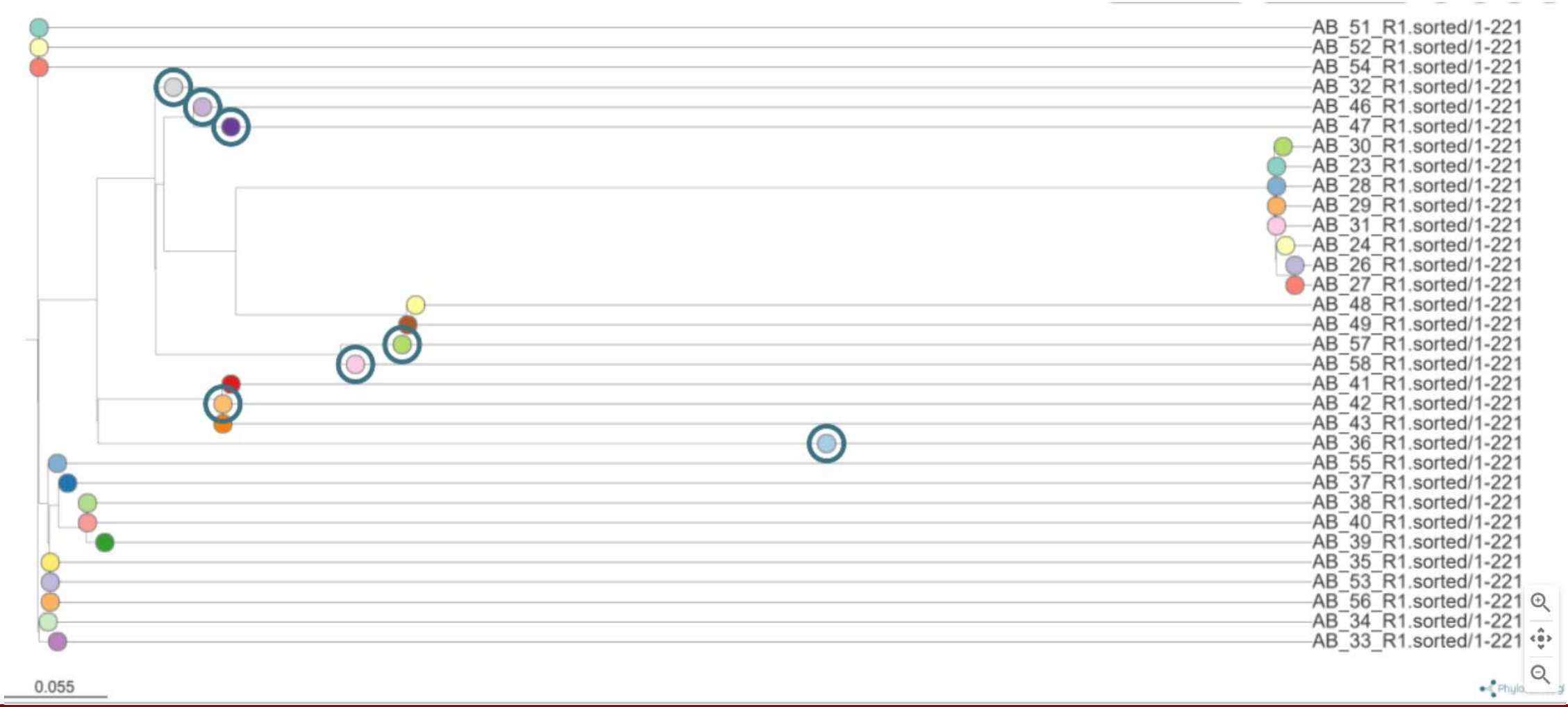
- Q6: There is a group of patients who have been traveling to country Z. Looking at the overall data for these, which scenarios did you identify?



I) AB32, AB_46 and AB_47 are closely related, but all three patients traveled to Z. There is no overlap of hospital admission, indicating sporadic travel-related cases.

Further patient 37 with AB_42 also traveled to Z with following intra-hospital transmission to AB_42 and AB_43

Survey 2 Q6 – Travel history Country Z



Survey 2 Question 7

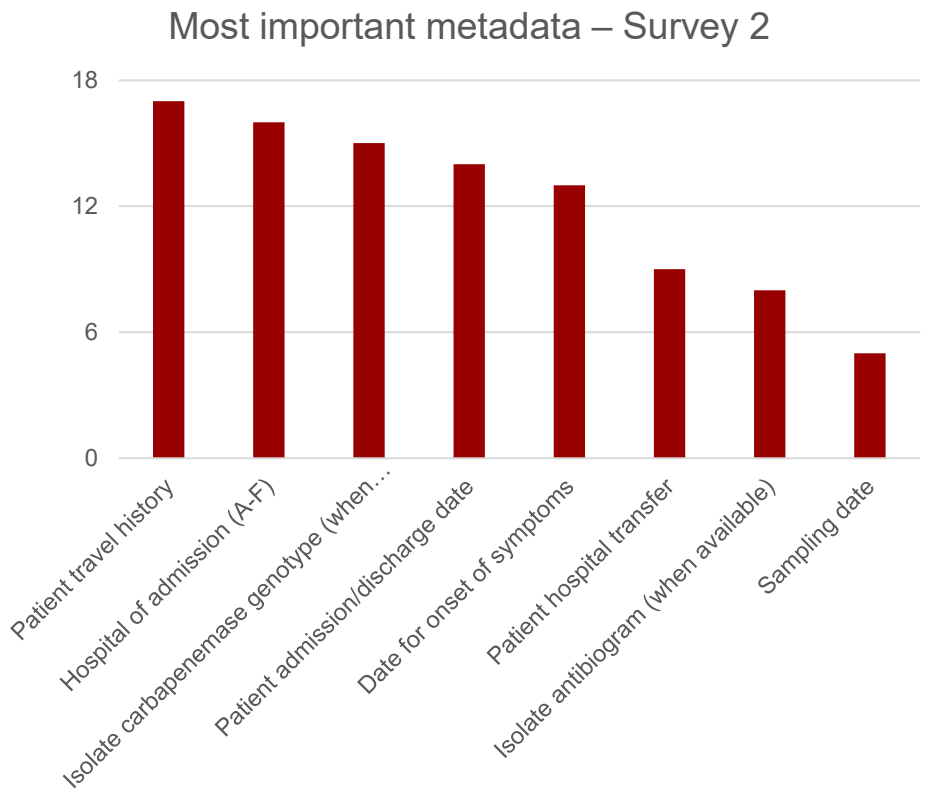
- Q7: There are two patients with a travel history related to Country W, but from different hospitals (patient 22 and 48; isolates AB_23 and AB_50)
- Assess if these two isolates seem to be related based on phylogenomic distance
  - YES(3), NO(15) (> 300 SNPs apart)

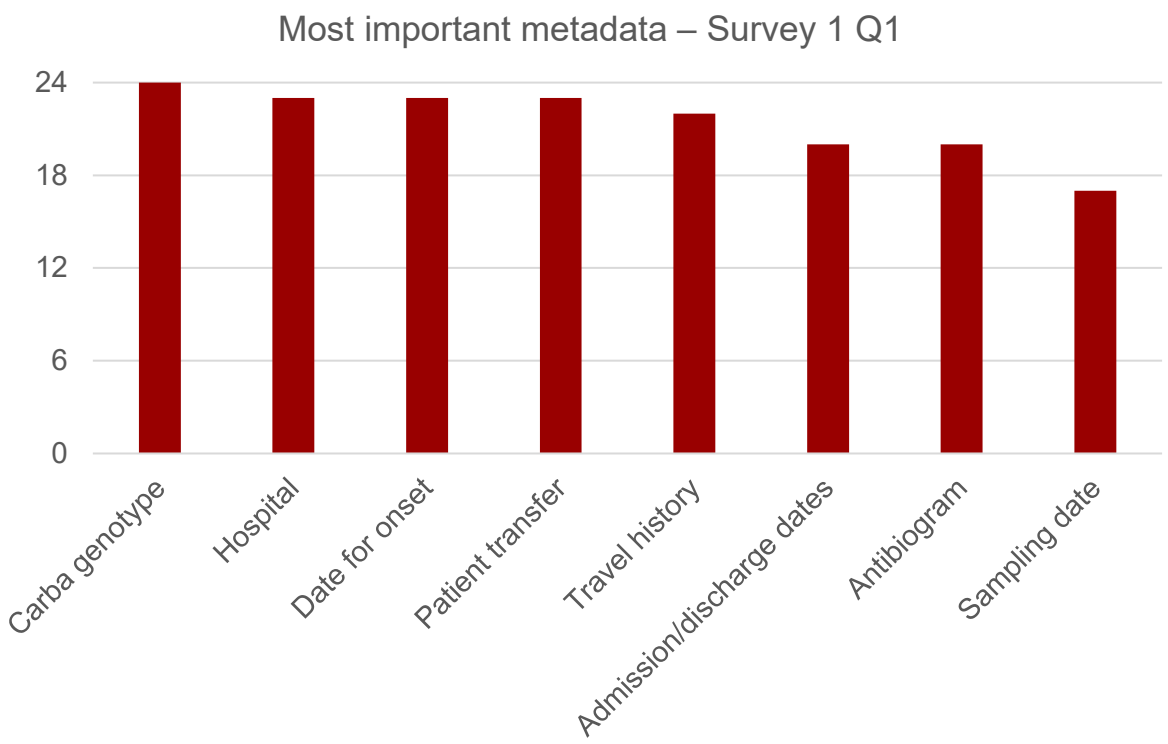
- Q7B: Check if the genotype responsible for the carbapenem phenotype is the same in these two isolates
  - On SeqSphere we saw that both have OXA-23 however they also have OXA-66 (AB23) and OXA-82(AB50)

- Q7C: Are any of these two strains part of a cluster/ an outbreak?
  - Patient no. 22 (AB_23) is part of a bigger cluster
- Q7D: Can you trace back the apparent origin of this outbreak
  - The first case is patient 22; travel history from country W, their hospitalization date is the earliest in the cluster, and each next isolate in the cluster overlaps the admission/discharge date.
  - The other metadata (phylogeny, antibiogram, MLST, cgMLST, genotype) support this hypothesis.

Survey 2 Question 8

- Which metadata indicators were in your opinion most important when selecting isolates for sequencing?





Survey 2 Question 9 - MLSTs and cgMLST

Q9: –what can you conclude on the two MLST schemes and the cgMLST data, in relation to their ability to discriminate between different outbreak isolates?

- MLST-Pasteur is less discriminative than MLST-Oxford and cgMLST is even more discriminative than MLST-Oxford.

- The discriminatory power of MLST-Pasteur is not good enough to identify the outbreak clusters, but those isolates that differed in this scheme did not belong to a cluster with others and could be excluded from further analysis on this basis alone.
- MLST-Oxford seems to have better discriminatory power, but there were isolates that belonged to the same cluster based on cgMLST and SNP analysis, but were classified into different ST types based on MLST-Oxford. Therefore MLST-Oxford was not found to be reliable. The cgMLST correctly identified several clusters, but the largest cluster identified by SNP analysis included 4 different cgMLST
- None of the methods can be used alone to identify outbreaks. SNP or allelic differences are important to know the relationship between the isolates.

Sum-up

Overall conclusion on the outbreak:
All types of outbreaks

Travel related outbreak(s)

Inter-hospital transmission(s)

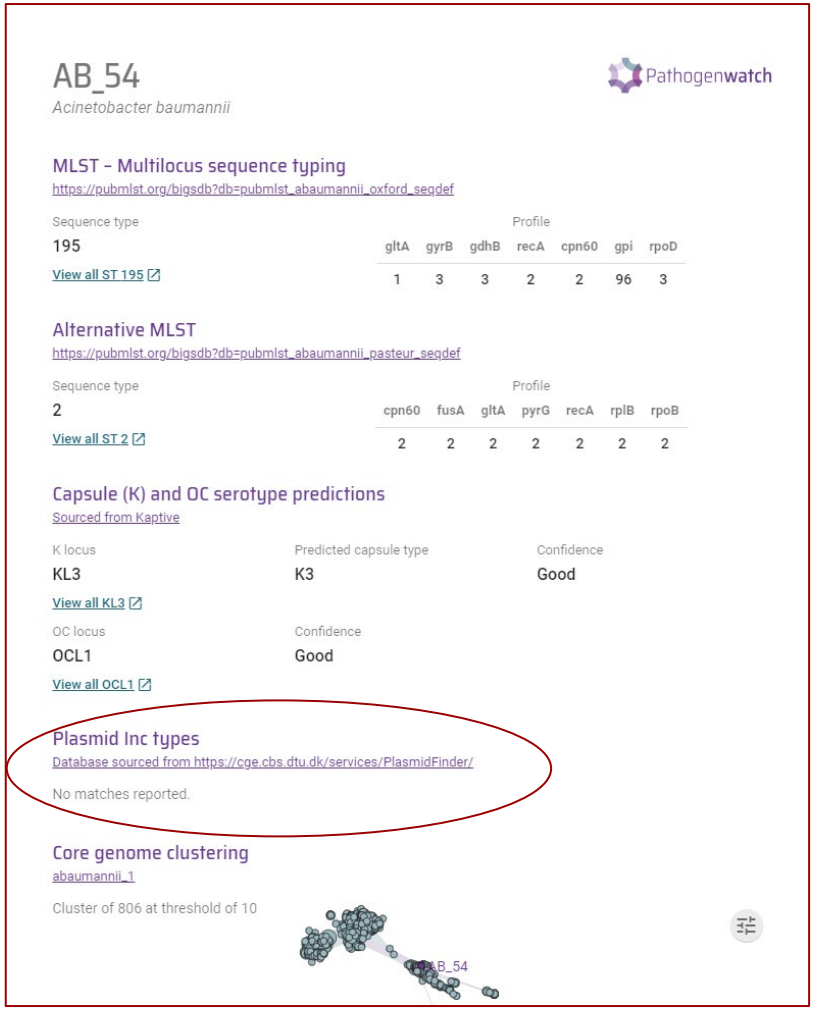
Intra-hospital transmission(s)

From Survey 1

- Could the metadata immediately suggest any of the below listed outbreak hypotheses?
  - Travel related outbreak(s)
  - Inter-hospital transmission(s)
  - Intra-hospital transmission(s) **(1)**
  - **A combination of the above (22)**
  - Data does not suggest any hypothesis **(1)**

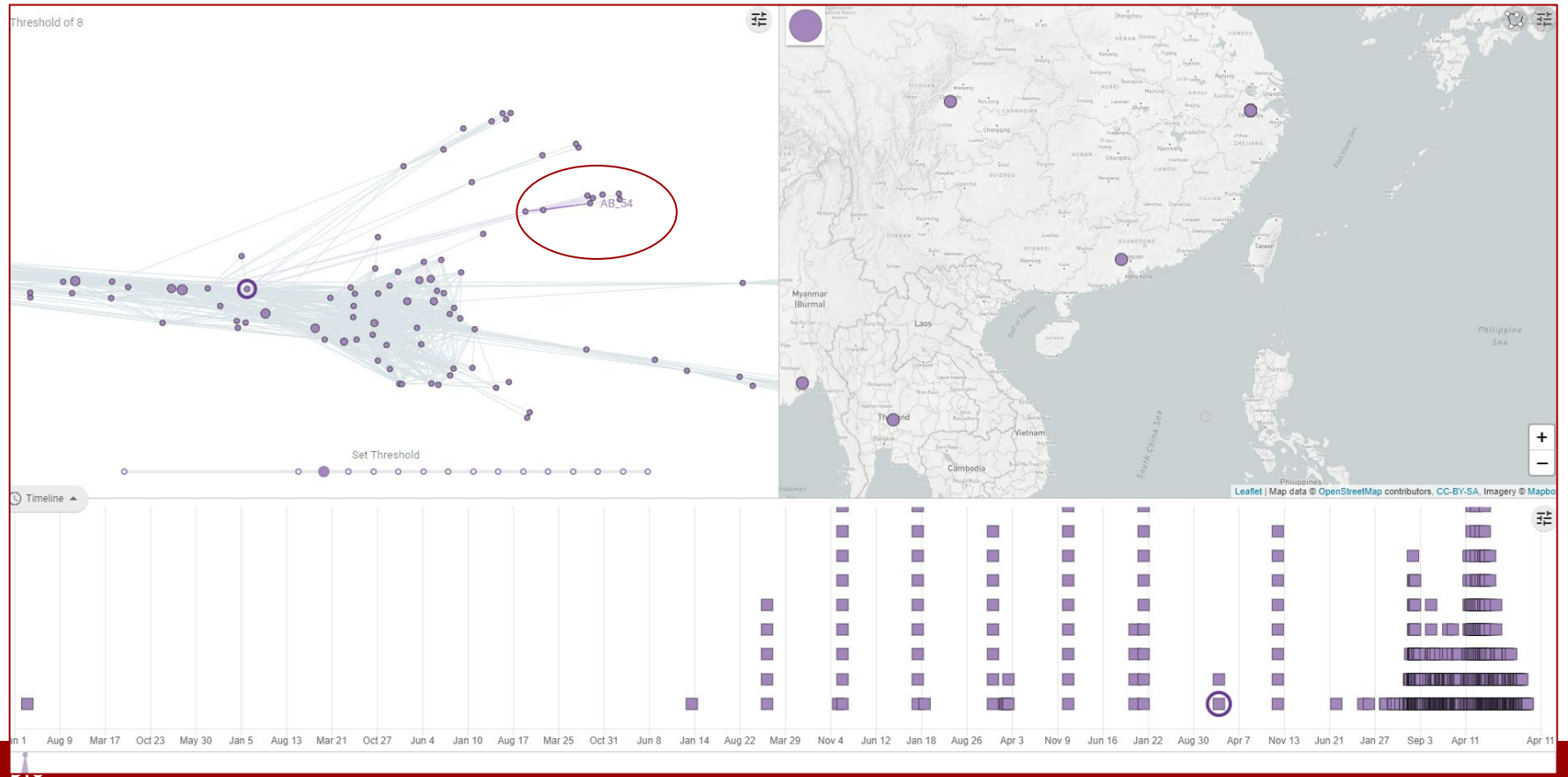
Additional analysis and typing of *A. baumannii*

- PathogenWatch (https://pathogen.watch/)
  - MLST (Oxford and Pasteur schemes)
  - Capsule Polysaccharide (K) typing (Kaptive)
  - LPS outer core (OCL) typing (Kaptive)
  - cgMLST based clustering
  - No plasmid replicon database for *A. baumannii*



Additional analysis and typing of *A. baumannii*

- PathogenWatch (https://pathogen.watch/)
  - cgMLST based clustering



Questions/Comments?